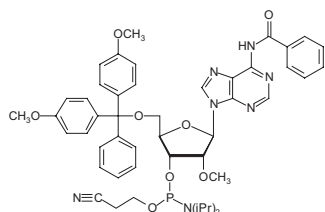


User Instructions

# 2'O-Methyl Phosphoramidites



**2'O-Methyl-rA(bz) Phosphoramidite**

**Product Description**

Chemical Formula:  $C_{48}H_{54}N_7O_8P$

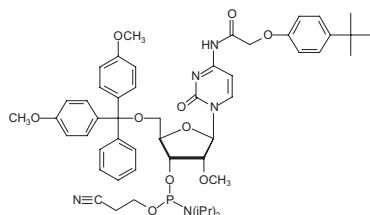
Formula Weight: 888.0

Storage: 4°C

DMT-2'O-Methyl-rAdenosine(N6-Benzoyl)-β-Cyanoethylphosphoramidite

**Product List**

A211181-01 0.5g PE™ 8900 and Polygen™ compatible  
A211131-01 0.5g ABI™ compatible  
A211110-01 10g Bulk



**2'O-Methyl-rC(tac) Phosphoramidite**

**Product Description**

Chemical Formula:  $C_{52}H_{64}N_5O_{10}P$

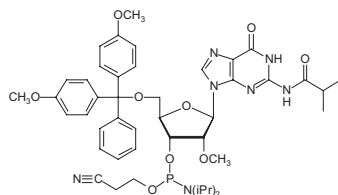
Formula Weight: 950.1

Storage: -20°C

DMT-2'O-Methyl-rCytidine(N4-tac)-β-Cyanoethylphosphoramidite

**Product List**

C212181-01 0.5g PE 8900 and Polygen™ compatible  
C212131-01 0.5g ABI compatible  
C212110-01 10g Bulk



**2'O-Methyl-rG(ib) Phosphoramidite**

**Product Description**

Chemical Formula:  $C_{45}H_{56}N_7O_9P$

Formula Weight: 870.0

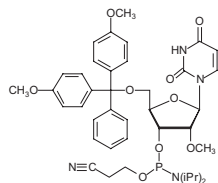
Storage: 4°C

DMT-2'O-Methyl-Guanosine(N2-Isobutyryl)-β-Cyanoethylphosphoramidite

**Product List**

G211181-01 0.5g PE 8900 and Polygen™ compatible  
G211131-01 0.5g ABI compatible  
G211110-01 10g Bulk

# 2'O-Methyl Phosphoramidites



**2'O-Methyl-rU Phosphoramidite**

## Product Description

Chemical Formula:  $C_{40}H_{49}N_4O_9P$

Formula Weight: 760.8

Storage: 4°C

DMT-2'O-Methyl-rUridine-β-Cyanoethylphosphoramidite

## Product List

U211181-01	0.5g PE 8900 and Polygen™ compatible
U211131-01	0.5g ABI compatible
U211110-01	10g Bulk

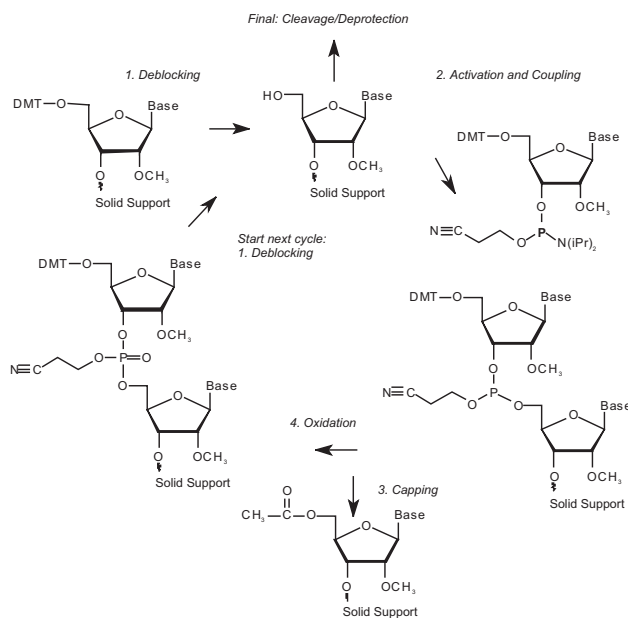
## 2'O-Methyl RNA Synthesis

The synthesis cycle for 2'O-methyl-oligoribonucleotides consists of the same series of reactions as the cycle that is employed for DNA monomers. However, the rate of coupling for 2'O-methyl RNA monomers is slower compared to that of DNA monomers (a coupling time of 6 minutes is recommended for 2'O-methyl RNA monomers compared to

90 seconds for DNA monomers). With the exception of the 2'O-methyl RNA monomers and supports, RNA synthesis is accomplished with the same reagents as DNA synthesis.

All 2'O-methyl RNA phosphoramidites from Proligo® Reagents are diluted with dry acetonitrile.

## The Synthesis Cycle



# 2'O-Methyl Phosphoramidites



## 2'O-Methyl RNA Monomers

2'O-methyl RNA monomers feature methoxy groups at the 2'-position. The methoxy groups are perfectly stable in all conditions employed in the assembly of oligonucleotides by automated phosphoramidite synthesis, and in all standard alkaline deprotection conditions.

## Base Protection

Proligo Reagents' 2'O-methyl RNA monomers are compatible with fast deprotection schemes that are based on the application of aliphatic amines, such as methylamine. The adenosine and guanosine monomers are protected with the standard benzoyl and isobutyryl groups. The uridine monomer is unprotected at the base, and the cytidine monomer is protected with a TAC (tert-butylphenoxyacetyl) group. This protecting group avoids transamination side reactions at cytidines, when alkylamines are employed in the deprotection reaction. AMA reagent (concentrated ammonia/40% aqueous methylamine 1/1, v/v) can be conveniently applied.

## 2'O-Methyl RNA Supports

The supports for 2'O-methyl oligoribonucleotide synthesis consist of a 2'O-methyl RNA nucleoside covalently attached through the 3'-position to controlled pore glass (CPG). The pore size of Proligo Reagents' CPG for 2'O-methyl oligoribonucleotide synthesis is 500Å. Proligo Reagents offers ready-to-use synthesis columns for 2'O-methyl oligoribonucleotide synthesis at a 1mmol scale.

## Methods

1. Use anhydrous acetonitrile (water content < 30ppm) as diluent. It is important to maintain anhydrous conditions while dissolving RNA phosphoramidites in acetonitrile.
2. For use on PE 8900 instruments, add 10ml of acetonitrile to 0.5g 2'O-methyl RNA monomer, to obtain a concentration of 50mg/ml. For use on PE 390 series instruments, add 5ml acetonitrile to 0.5g 2'O-methyl RNA monomer, to obtain a concentration of 100mg/ml.
3. Gently swirl the vial until the powder is completely dissolved.
4. Attach the dissolved phosphoramidite to the appropriate position on the synthesizer. Ensure that the delivery line to the synthesis chamber is sufficiently primed.
5. Enter the sequence of the 2'O-methyl oligoribonucleotide you wish to synthesize. A minimum coupling time of 6 minutes is recommended for 2'O-methyl RNA phosphoramidites.
6. Proceed as you would with a standard DNA oligonucleotide synthesis. Depending on your intended further use of the oligomer, you can choose either DMT-On or DMT-Off procedures. The coupling efficiency of 2'O-methyl RNA monomers may be determined by standard dimethoxytrityl cation assays.



# 2'O-Methyl Phosphoramidites



7. Cleave from the support and deprotect the 2'O-methyl oligoribonucleotide with concentrated ammonia at 55°C for 8 hours. Alternatively, AMA reagent (concentrated ammonia/40% aqueous methylamine 1/1, v/v) can be employed for 10 minutes at 65°C.

8. The 2'O-methyl oligoribonucleotide is now ready for further processing, such as desalting or purification with RP-HPLC, AX-HPLC or gel-based methods. Purification of fully-modified 2'O-methyl RNA oligonucleotides is simpler than in case of RNA, as no special precautions are required to prevent nucleolytic degradation.

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